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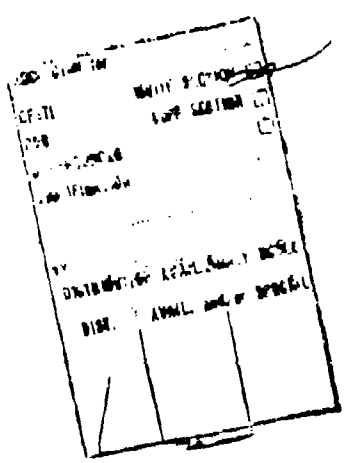

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FOREWORD

The work described in this paper was accomplished in the Radiobiology Branch under task No. 776703. The work was performed from November 1965 through March 1966. The paper was submitted for publication on 19 July 1967

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

Azathioprine was obtained from Burroughs Wellcome and Co., Inc., Tuckahoe, N.Y. The ICD analyses utilized a commercially available kit, Dermatube-ICD, Worthington Biochemical Corp., Freehold, N.J. SGPT analyses are currently performed using a commercially available kit from Dade Reagents, Inc., Miami, Fla. Protein electrophoresis was performed using a Beckman Microzone Electrophoresis System, Beckman Instruments, Inc., Fullerton, Calif.

The authors thank Dr. P. Crump and R. McNee of the Biometrics Branch for statistical support; Major H. Casey and Captain J. Woodruff for interpretation of the histologic sections; Staff Sergeant B. Kennon and Airman First Class H. Samuels of the Veterinary Sciences Division for technical support; and Airman First Class G. Ford for his assistance in many aspects of this project.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

Forty-five rhesus monkeys received 980 R whole-body irradiation from a cobalt-60 source followed by allogeneic bone marrow transplants. The animals were randomly divided into three groups: (1) bone marrow, no drug; (2) bone marrow, azathioprine; and (3) bone marrow, amethopterin. Supportive care was the same for all groups. Although there was a statistically significant prolongation of the average survival time in the group receiving amethopterin, the net effect was one of briefly delaying the onset of secondary disease as the clinical course of these animals ultimately paralleled that of the other groups. There was no significant difference in the average survival time of the azathioprine group as compared with the control group, and by the criteria cited there were fewer takes effected in the animals receiving azathioprine. The two drugs tested were essentially ineffective in the dose levels and regimens utilized.

EFFECT OF AZATHIOPRINE AND AMETHOPTERIN ON SECONDARY DISEASE IN THE RHESUS MONKEY

I. INTRODUCTION

Considerable species variation exists in the course of secondary disease after postirradiation allogeneic bone marrow transplantation. In mice late mortality may range up to 100%, and in dogs a clinically severe syndrome has been described by Thomas et al. (22, 23) and Cole and Alpen (7). Other species have been shown to manifest secondary disease, and the species differences have been summarized by Van Putten (21). In primates successful postirradiation allogeneic bone marrow transplantation is followed by the rapid onset of a severe and uniformly fatal form of secondary disease. The clinical syndrome and pertinent pathologic findings have been well documented in the rhesus monkey (8, 9, 10). The similarity between the course of secondary disease in the monkey and man provides a potentially useful model to evaluate the ability of various immunosuppressive agents to modify secondary disease in humans (5).

Uphoff (26) in 1958 showed that amethopterin was effective in altering the course of secondary disease in mice; this finding was later confirmed by Lochte et al. (14). Thomas and his associates (23, 24, 25) have shown that in dogs receiving allogeneic bone marrow transplants it is possible to increase the survival time by treatment with amethopterin. Treatment regimens included administration of amethopterin both pre- and posttransplantation. There is limited information available in the literature on the effect of immunosuppressive agents on the course of secondary disease in the rhesus monkey. Van Putten (27) in 1964 reported preliminary results using amethopterin and azathioprine and suggested that the drugs might prove useful. Since the completion of this study, more information has become available. Muller-Berat et al. (19) and Van Bekkum (5) have reported some success in modifying secondary disease in monkeys by use of amethopterin and cyclophosphamide, with a greater degree of success using the latter drug.

The ability of azathioprine to suppress immune responses in humans after organ transplants and the effectiveness of amethopterin in attenuating secondary disease in rodents and dogs prompted a trial of these drugs in the rhesus monkey.

II. MATERIALS AND METHODS

Forty-five rhesus monkeys (27 males and 18 females), weighing 2.4 to 3.5 kg., were divided into 15 sets of 3 animals each. In each set the animals were of similar weights and identical sex and red cell type. Erythrocytic typing in the rhesus monkey has been previously described by Owen and Anderson (20). Animals within each set were randomly assigned to one of the two drug groups or the control group. The animals were used as both donors and recipients, and animals within each set were randomly matched for this purpose.

The animals were lightly anesthetized with intravenous sodium thiopental; both buttocks and thighs were shaved and prepared for surgery, and a stab wound was made over the greater trochanter of one of the femurs. A hole was drilled through the trochanteric fossa using a Steinmann pin ($^3/_{32}$ or $^1/_8$ inch) in a hand chuck. This route of obtaining bone

marrow specimens from the rhesus monkey has been described by several authors (1, 12). A Vim-Silverman needle (3⅝ inches) was inserted into the marrow cavity and the marrow aspirated by advancing and retracting the needle several times along the length of the marrow cavity. The needle was connected to a vacuum source through a three-way stopcock, which allowed the marrow to be intermittently mixed with heparinized TC-199 medium (Difco) during aspiration. After the marrow was obtained from one femur, the animal was turned and the procedure repeated on the opposite side. This technic is relatively simple, and it is possible to aspirate the marrow from the femurs of 8 monkeys in 1½ to 2 hours. By use of this method, the transplants were performed four days a week for a period of two weeks.

The vacuum apparatus is shown in figure 1. The vacuum pump was connected to a tank, which served to dampen any marked pressure changes within the system; the pressure was held at approximately ⅓ atmosphere. A bleeder valve allowed the pressure to be increased above the minimum obtained with the pump alone, if desired. A sidearm flask served as a trap to exclude any fluid accidentally aspirated beyond the plastic tube used for marrow collection. The plastic tube was removed when partially filled; additional media were added; then the tube was capped with a perforated top and stored in ice. Connections between the pump, tank, and other points within the system were made with Tygon tubing.

After aspiration, the marrow was mixed with an additional 30 ml. of TC-199 medium to a final volume of approximately 90 ml. Heparin (10,000 U.S.P. units), 32,000 units of penicillin, and 40 mg. of streptomycin were added to each liter of TC-199 medium. The aspirated marrow mixture was stored in ice until centrifugation, which was performed in a refrigerated centrifuge (2° to 4° C.) for 20 minutes at 170 × g. The majority of the supernatant fluid and fat was aspirated and the marrow resuspended by aspiration into a syringe through a 20-gage needle, which also served to remove any small clots and bone chips. No other filtration or screening was utilized. The

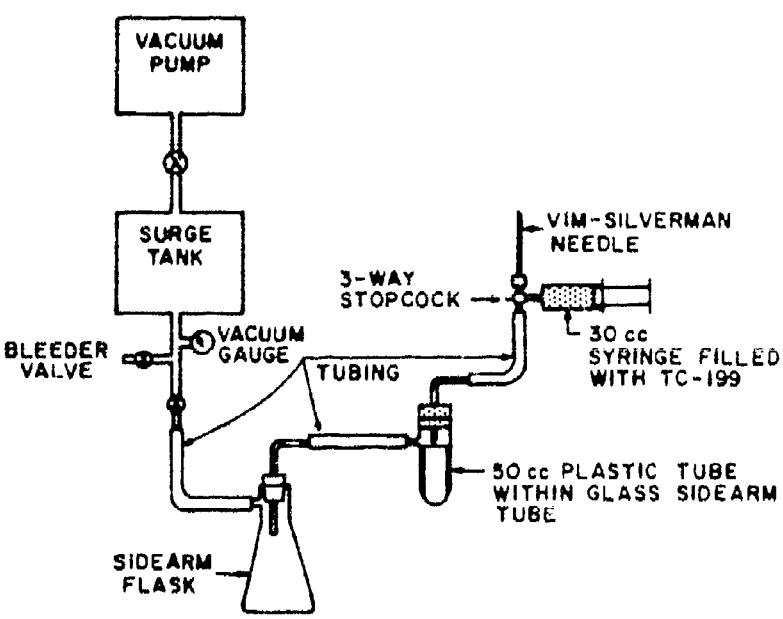


FIGURE 1

Vacuum apparatus used for marrow extractions.

final marrow suspension constituted a volume of 20 to 30 ml. and was stored at 2° to 4° C. until reinfusion, a period of 2 to 3 hours. Total storage time for the aspirated marrow was 4 to 6 hours.

After a 2- to 3-hour recovery from anesthesia, the animals were irradiated using a cobalt-60 facility, the details of which have been previously described (13). The animals were irradiated in a wooden box using anterior and posterior fields; they were turned halfway through the period of irradiation, thus requiring a 1- to 2-minute interruption. A midline air dose of 980 R was delivered at 98 R per minute. Approximately 1 to 2 hours postirradiation the animals were again lightly anesthetized with intravenous sodium thiopental, and marrow from the appropriate donor was infused in a leg vein. The marrow was injected over a period of 1 minute. There was no direct morbidity or mortality associated with the transplantation procedure. Prophylactic antibiotics were administered daily from days 2 to 15 posttransplantation and consisted of 300,000 units of penicillin and 40 mg. of tetracycline intramuscularly once a day. Preliminary work using this transplantation procedure in animals receiving autologous transplants after a midline air dose of 930 or 980 R under similar conditions resulted in takes in all 14 animals. Two animals subsequently died at 28 and 37 days; both deaths may have been secondary to drugs which the animals were receiving, as the peripheral blood counts had returned to normal before the animals died. The remaining 12 animals are alive 19 months after transplantation.

Since the marrow was obtained from living donors, the suspensions consisted of a mixture of bone marrow and blood and the cell counts have been expressed as "total nucleated cells." The number of cells received ranged from 4.1×10^8 to 2.2×10^9 total nucleated cells (1.2×10^8 to 6.7×10^8 nucleated cells per kilogram). Those animals receiving immunosuppressive agents were given the initial dose at the time of the marrow infusion (day 0). Azathioprine (3 mg./ml.) was administered intravenously in a dose of 3.0 mg./kg. on days 0, 1, and 2, and 1.0 mg./kg. on days 3 through 14 posttransplantation. Amethopterin (5 mg./ml.) was administered in four subcutaneous sites each day on days 0, 2, 4, 6, and 8 posttransplantation in a dose of 1.0 mg./kg. A summary of pertinent transplantation data for each animal is presented in table I.

All animals were housed in individual, adjacent wire cages with free access to water. Routine feeding consisted of a commercial monkey chow interspersed with peanuts and fruit. Daily weights and temperatures were obtained on each animal as well as evaluation of activity, appetite, appearance, and elimination. If an animal was found dead in the morning, survival was scored until the preceding day. All animals were necropsied, and the histopathologic findings will be reported separately.

Baseline blood samples were obtained on two occasions in the six weeks before transplantation; the second sample was obtained approximately two weeks before transplantation and has been presented in the following data as the baseline value. Posttransplantation, the animals were sampled twice weekly for peripheral blood counts and once per week for serum enzyme and chemistry determinations. The initial sample was obtained on day 7 with subsequent samples on day 10 or 11 (these points have been pooled in the data and presented as an 11-day point), day 14, and in the few survivors, on days 17 and 21. In addition, counts were occasionally performed if an animal was extremely ill or moribund to determine if there had been a take. Since most of the postirradiation data does not follow a normal distribution, the data have been calculated and tabulated as the 25th, 50th, and 75th percentiles.

The peripheral blood counts included hematocrit, platelet, reticulocyte, and leukocyte counts; serum chemistry and enzyme determinations included isocitric dehydrogenase (ICD), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phophatase, lactic dehydrogenase, leucine aminopeptidase (LAP),

TABLE I

Summary of transplantation data

Set and red cell type	Group	Total cells received	Number of cells/kg.	Take	Survival (days)
1	Control	1.9×10^{9}	6.6×10^{7}	Yes	12
ABF	Amethopterin	8.9×10^{8}	3.0×10^{7}	Yes	21
	Azathioprine	5.2×10^{8}	1.7×10^{7}	No	8
2	Control	1.0×10^{9}	3.6×10^{7}	No	9
ABCF	Amethopterin	9.8×10^{8}	3.4×10^{7}	Yes	15
	Azathioprine	1.0×10^{9}	3.6×10^{7}	No	8
3	Control	5.5×10^{8}	2.1×10^{7}	No	6
BDF	Amethopterin	1.2×10^{9}	3.9×10^{7}	Yes	17
	Azathioprine	1.5×10^{9}	4.7×10^{7}	Yes	11
4	Control	8.4×10^{8}	2.8×10^{7}	No	7
BDF	Amethopterin	5.7×10^{8}	1.8×10^{7}	Yes	19
	Azathioprine	8.0×10^{8}	3.3×10^{7}	Yes	10
5	Control	4.5×10^{8}	1.7×10^{7}	Yes	8
BCF	Amethopterin	6.0×10^{8}	2.3×10^{7}	Yes	17
	Azathioprine	5.4×10^{8}	2.1×10^{7}	No	14
6	Control	2.2×10^{9}	6.7×10^{7}	Yes	8
BCF	Amethopterin	1.3×10^{9}	4.1×10^{7}	Yes	14
	Azathioprine	1.8×10^{9}	5.1×10^{7}	Yes	11
7	Control	8.4×10^{8}	3.0×10^{7}	Yes	12
BF	Amethopterin	8.4×10^{8}	2.7×10^{7}	No	7
	Azathioprine	1.5×10^{9}	4.5×10^{7}	Yes	13
8	Control	1.6×10^{9}	6.4×10^{7}	Yes	13
BF	Amethopterin	1.1×10^{9}	3.9×10^{7}	Yes	15
	Azathioprine	1.4×10^{9}	5.4×10^{7}	Yes	12
9	Control	1.0×10^{9}	3.4×10^{7}	Yes	14
BEF	Amethopterin	1.5×10^{9}	6.3×10^{7}	Yes	15
	Azathioprine	6.1×10^{8}	2.2×10^{7}	No	8
10	Control	1.4×10^{9}	4.2×10^{7}	Yes	13
BEF	Amethopterin	1.3×10^{9}	4.1×10^{7}	Yes	15
	Azathioprine	1.1×10^{9}	4.1×10^{7}	No	9
11	Control	5.0×10^{8}	1.7×10^{7}	Yes	11
BF	Amethopterin	9.7×10^{8}	3.1×10^{7}	Yes	15
	Azathioprine	9.1×10^{8}	2.8×10^{7}	No	13
12	Control	1.0×10^{9}	3.3×10^{7}	Yes	13
BF	Amethopterin	8.0×10^{8}	2.9×10^{7}	No	12
	Azathioprine	5.8×10^{8}	2.0×10^{7}	Yes	10
13	Control	4.1×10^{8}	1.2×10^{7}	No	8
BF	Amethopterin	1.1×10^{9}	3.1×10^{7}	No	9
	Azathioprine	1.2×10^{9}	4.1×10^{7}	No	8
14	Control	9.2×10^{8}	3.3×10^{7}	Yes	12
BCF	Amethopterin	1.5×10^{9}	4.7×10^{7}	No	13
	Azathioprine	4.4×10^{8}	1.4×10^{7}	No	7
15	Control	1.1×10^{9}	4.2×10^{7}	Yes	11
BF	Amethopterin	1.0×10^{9}	3.6×10^{7}	Yes	17
	Azathioprine	1.2×10^{9}	4.8×10^{7}	Yes	12

blood urea nitrogen (BUN), creatinine, and total protein with electrophoresis. The methods of determination and normal values for the rhesus monkey in our laboratory have been previously reported by Anderson (3) with the exception of the isocitric dehydrogenase.

III. RESULTS AND DISCUSSION

Several authors have demonstrated the ability of transplanted allogeneic bone marrow to proliferate in lethally irradiated monkeys (1, 15, 19, 27, 28). In earlier work from our laboratory, the erythrocytic types in donor and recipient animals were purposely mismatched in order to confirm that a take had been established (27, 28). In the present study, since each set was of the same erythrocytic type, it was not possible to use this method of assessing a take. In addition, since the animals in each set were of the same sex, it was not possible to evaluate the presence of female donor cells in males by karyotype or by using drumstick markers as described by Magliulo et al. (15). We therefore defined a *take* as an increasing reticulocyte count ($\geq$ 0.3%) or an increasing leukocyte count ($\geq$ 1,000 cells) per cubic millimeter, or both. Justification for this was based on the results obtained from two preliminary groups of animals in which: (1) animals receiving autologous transplants under the above conditions showed beginning recovery of their peripheral blood elements by 10 days posttransplantation; and (2) 10 animals irradiated under the above conditions but not receiving bone marrow transplants showed no evidence of recovery of either reticulocyte or leukocyte counts before death, which occurred from 10 to 16 days postirradiation, with a median of 12 days. Pertinent data from this group have been included for purposes of comparison.

By use of these criteria to define a take, there were 73% takes in both the control and amethopterin groups and 47% in the azathioprine group (table II). These numbers probably represent minimal figures, as some animals died before the time that a take was evident from the peripheral blood counts.

The hematologic data for all groups have been tabulated in table III. The hematocrits declined in all groups postirradiation, as shown in figure 2. The leukocyte and reticulocyte counts (fig. 3) were markedly reduced at day 7, as were the platelet counts (fig. 2), although the group receiving no bone marrow showed a less marked platelet drop at 7 days, thus raising the possibility that the graft-versus-host reaction in the animals receiving allogeneic bone marrow transplants contributed to platelet destruction. The group receiving only bone marrow showed an increase in the leukocyte, platelet, and reticulocyte counts at day 11. The azathioprine group showed a similar increase in platelets by day 11, but no change in the leukocyte count at that time; the reticulocyte count was also increasing at day 11, but not as

TABLE II

Percent takes

Group*	Reticulocytes $\geq$ 0.3%		WBC $\geq$ 1,000/mm.^{3}		Total takes	
	Number	Percent	Number	Percent	Number	Percent
Control	9	60	11	73	11	73
Azathioprine	7	47	1	6	7	47
Amethopterin	11	73	8	53	11	73

*Fifteen animals in each group.

FIGURE 2

Variation of hematocrit and platelet count after transplantation.

TABLE III

Hematocrit, platelets, leukocytes, reticulocytes tabulated in percentiles: 50th (25th, 75th)

Day	Irradiation only	Bone marrow, no drug	Bone marrow, azathioprine	Bone marrow, amethopterin
		Hematocrit (%)		
Baseline	40.5 (39.0, 42.5)	39.0 (37.0, 41.0)	37.0 (35.0, 39.0)	38.0 (37.0, 40.0)
7	37.5 (36.0, 39.5)	35.0 (33.0, 38.0)	34.0 (32.0, 37.0)	37.0 (33.0, 39.0)
11	34.5 (26.5, 37.5)	33.0 (28.0, 38.0)	29.0 (24.0, 34.0)	33.5 (31.0, 34.0)
14	—	—	—	31.0 (28.0, 32.5)
		Platelets $\times 10^3$		
Baseline	344 (309, 368)	276 (236, 291)	265 (236, 352)	267 (248, 275)
7	186 (133, 205)	26 (17, 62)	20 (14, 58)	50 (40, 102)
11	22 (11, 29)	77 (60, 83)	65 (37, 80)	52 (24, 58)
14	—	—	—	98 (60, 136)
		Leukocytes $\times 10^3$		
Baseline	12.3 (11.5, 13.9)	9.20 (6.25, 10.9)	8.85 (6.60, 10.9)	7.20 (5.90, 8.45)
7	0.28 (0.22, 0.40)	0.45 (0.25, 0.65)	0.35 (0.15, 0.55)	0.10 (0.05, 0.20)
11	0.11 (0.05, 0.17)	2.00 (1.00, 2.30)	0.40 (0.28, 0.73)	0.25 (0.15, 0.40)
14	—	—	—	1.06 (0.63, 1.59)
		Reticulocytes (%)		
Baseline	0.5 (0.4, 0.5)	0.7 (0.4, 0.9)	0.5 (0.3, 0.7)	0.5 (0.3, 1.1)
7	0	0	0	0
11	0	1.0 (0.6, 2.4)	0.4 (0.05, 1.00)	0.1 (0, 0.2)
14	—	—	—	0.5 (0.4, 2.5)

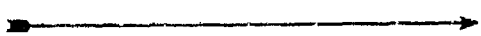
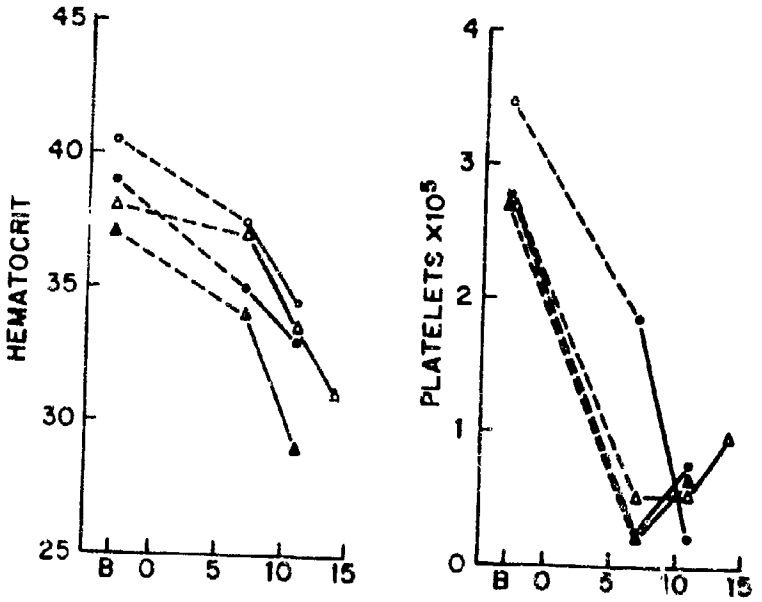


FIGURE 3

Variation of leukocyte and reticulocyte counts after transplantation.

KEY:

B = BASELINE
o = IRRADIATION ONLY
• = BONE MARROW, CONTROL
▲ = BONE MARROW, AZATHIOPRINE
Δ = BONE MARROW, AMETHOPTERIN



KEY:

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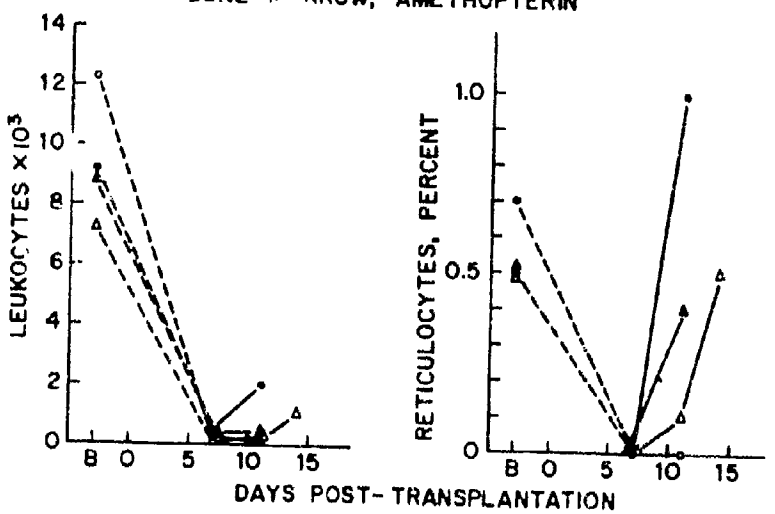

TABLE IV

ICD, SGOT, SGPT, and LDH tabulated in percentiles: 50th (25th, 75th)

Day	Irradiation only	Bone marrow, no drug	Bone marrow, azathioprine	Bone marrow, amethopterin
		ICD		
Baseline	10 (8, 17)	14 (10, 21)	14 (11, 19)	18 (10, 17)
7	11 (9, 14)	54 (31, 64)	60 (36, 89)	17 (13, 24)
14	—	—	—	41 (24, 62)
		SGOT		
Baseline	36 (29, 40)	26 (18, 33)	28 (22, 32)	25 (20, 28)
7	23 (21, 26)	65 (32, 115)	76 (53, 97)	51 (42, 64)
14	—	—	—	50 (43, 96)
		SGPT		
Baseline	19 (19, 22)	27 (21, 30)	25 (23, 30)	22 (20, 31)
7	27 (23, 29)	66 (42, 91)	76 (55, 105)	52 (32, 74)
14	—	—	—	64 (41, 96)
		LDH		
Baseline	555 (390, 680)	415 (360, 510)	395 (320, 565)	430 (340, 465)
7	448 (385, 485)	1,088 (715, 1,338)	1,145 (840, 1,395)	815 (550, 950)
14	—	—	—	653 (565, 1,060)

TABLE V

Leucine aminopeptidase (LAP), alkaline phosphatase, BUN, and creatinine tabulated in percentiles: 50th (25th, 75th)

Day	Irradiation only	Bone marrow, no drug	Bone marrow, azathioprine	Bone marrow, amethopterin
		LAP		
Baseline	190 (159, 209)	175 (147, 217)	177 (149, 221)	231 (173, 259)
7	171 (133, 185)	305 (169, 357)	250 (165, 412)	170 (158, 257)
14	—	—	—	113 (90, 226)
		Alkaline phosphatase		
Baseline	19 (16, 21)	14 (11, 17)	15 (12, 22)	15 (12, 19)
7	13 (11, 19)	7 (6, 9)	10 (6, 12)	8 (6, 11)
14	—	—	—	9 (6, 11)
		BUN (mg %)		
Baseline	19 (17, 21)	18 (16, 20)	19 (17, 20)	19 (17, 21)
7	21 (18, 34)	45 (34, 55)	46 (30, 95)	38 (23, 59)
14	—	—	—	33 (22, 45)
		Creatinine (mg. %)		
Baseline	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)
7	0.9 (0.7, 1.0)	1.0 (0.9, 1.1)	0.9 (0.7, 1.4)	0.8 (0.7, 0.9)
14	—	—	—	0.6 (0.5, 0.9)

sharply as in the group receiving no immunosuppressive agents. The group receiving amethopterin manifested a sustained suppression of the leukocyte and platelet counts at day 11; however, the counts increased by day 14; the reticulocyte counts showed a slight increase by day 11 and a further increase by day 14. The reticulocyte counts of all of the groups receiving transplants were increasing by day 11, showing the most marked increase in the group receiving no immunosuppressive agents. The reticulocyte, leukocyte, and platelet counts increased during the period of increasing mortality for each of the groups receiving bone marrow transplants.

The serum chemistry and enzyme determinations have been tabulated in tables IV, V, and VI, and the more significant results are shown in figure 4. The isocitric dehydrogenase (ICD) remained essentially unchanged at 7 days in the group receiving no bone marrow and in the amethopterin group. The control group and the azathioprine group showed a sharp and significant increase in this parameter at 7 days ($P < .01$). The SGOT and SGPT show parallel trends, but differ from the ICD determination at 7 days as there is a significant elevation in all of the groups receiving bone marrow transplants, while in the group receiving no bone marrow there is not a

TABLE VI

Total protein and electrophoresis results tabulated in percentiles: 50th (25th, 75th)

Day	Irradiation only	Bone marrow, no drug	Bone marrow, azathioprine	Bone marrow, amethopterin
		Total protein (gm. %)		
Baseline	7.6 (7.2, 8.0)	7.7 (7.3, 8.2)	8.1 (6.9, 8.3)	7.7 (7.4, 8.1)
7	6.9 (6.6, 7.1)	6.3 (5.9, 6.8)	6.3 (5.6, 6.6)	7.1 (6.8, 7.5)
14	—	—	—	5.8 (5.6, 6.2)
		Albumin (gm. %)		
Baseline	4.6 (4.4, 4.8)	4.5 (4.0, 4.8)	4.4 (4.2, 4.7)	4.9 (4.2, 5.1)
7	4.6 (4.4, 4.7)	3.1 (2.8, 3.5)	3.0 (2.8, 3.3)	3.7 (3.0, 4.2)
14	—	—	—	2.8 (2.3, 3.6)
		Alpha$_1$ and alpha$_2$ globulins (gm. %)		
Baseline	0.3 (0.3, 0.4)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.8 (0.7, 1.0)
7	0.6 (0.5, 0.6)	1.0 (0.8, 1.4)	1.1 (0.9, 1.5)	1.1 (0.9, 1.2)
14	—	—	—	0.9 (0.7, 1.3)
		Beta globulins (gm. %)		
Baseline	1.2 (0.9, 1.3)	1.3 (0.9, 1.5)	1.2 (1.0, 1.3)	1.0 (0.9, 1.3)
7	1.1 (0.9, 1.2)	1.3 (1.2, 1.6)	1.4 (1.3, 1.5)	1.5 (1.0, 1.7)
14	—	—	—	1.2 (1.1, 1.6)
		Gamma globulins (gm. %)		
Baseline	1.5 (1.3, 1.6)	1.0 (0.9, 1.2)	1.3 (0.8, 1.6)	1.1 (0.9, 1.3)
7	0.8 (0.6, 0.9)	0.7 (0.5, 0.8)	0.6 (0.5, 0.8)	0.8 (0.7, 1.0)
14	—	—	—	0.6 (0.3, 0.7)

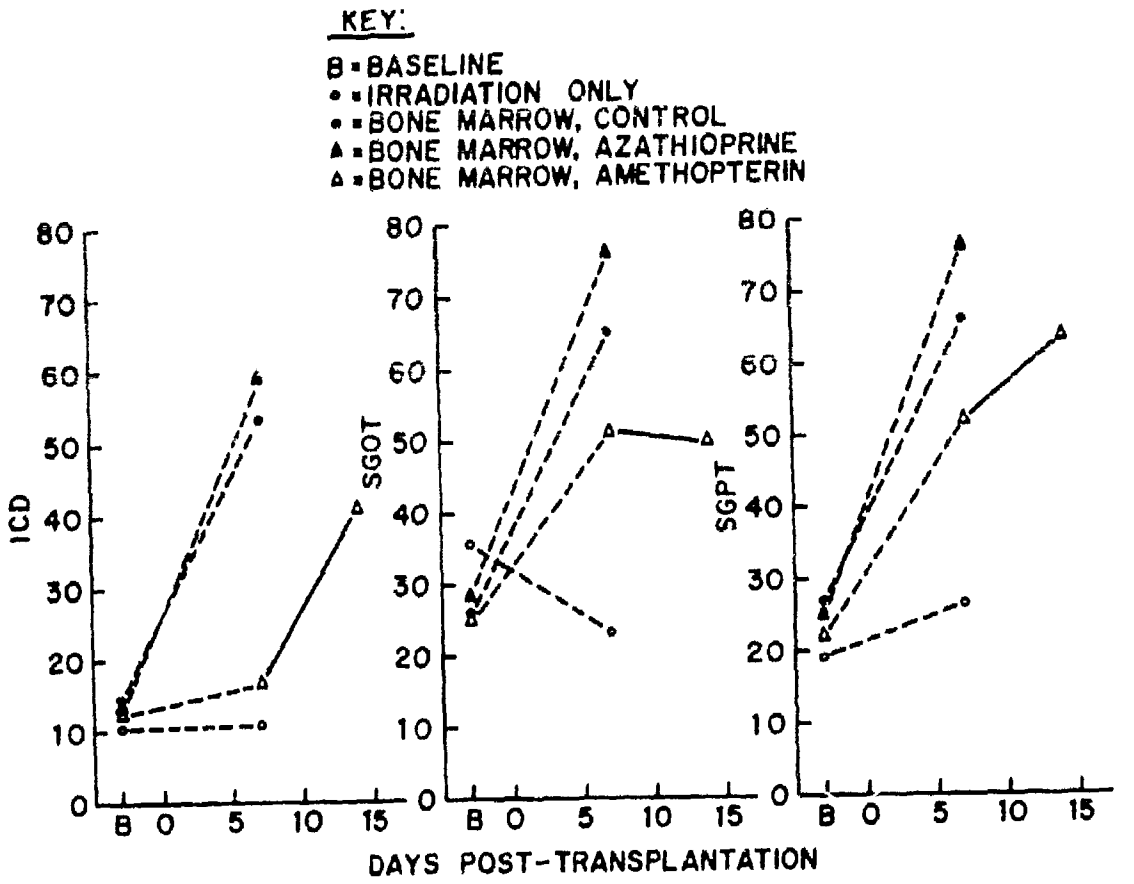

FIGURE 4

Variation of ICD, SGOT, and SGPT after transplantation.

significant difference between the baseline value and the 7-day value. This sharp increase in the SGOT, SGPT, and the ICD at 7 days is not seen in animals receiving autologous transplants (11 27). The ICD level is reported to be a sensitive measure for the determination of hepatic cell injury (2). In this study the group receiving amethopterin revealed no significant change in the ICD level at 7 days, while the SGOT and SGPT levels were elevated significantly ($P < .01$). At 14 days the ICD level increased ($P < .05$), while there was no significant change in the SGOT and SGPT levels as compared to the 7-day determinations.

Since the objective of the study was to maintain living animals either in the form of stable chimeras or as possible reversals, the parameter of greatest interest was survival. Examination of the survival curves (fig. 5) shows no significant difference between the curves for the control and azathioprine groups, with no survivors beyond 14 days. Two-thirds of the group treated with amethopterin were alive at day 14, but there were no survivors beyond 21 days.

A more meaningful comparison is that of the average survival time, which was 10.5 days

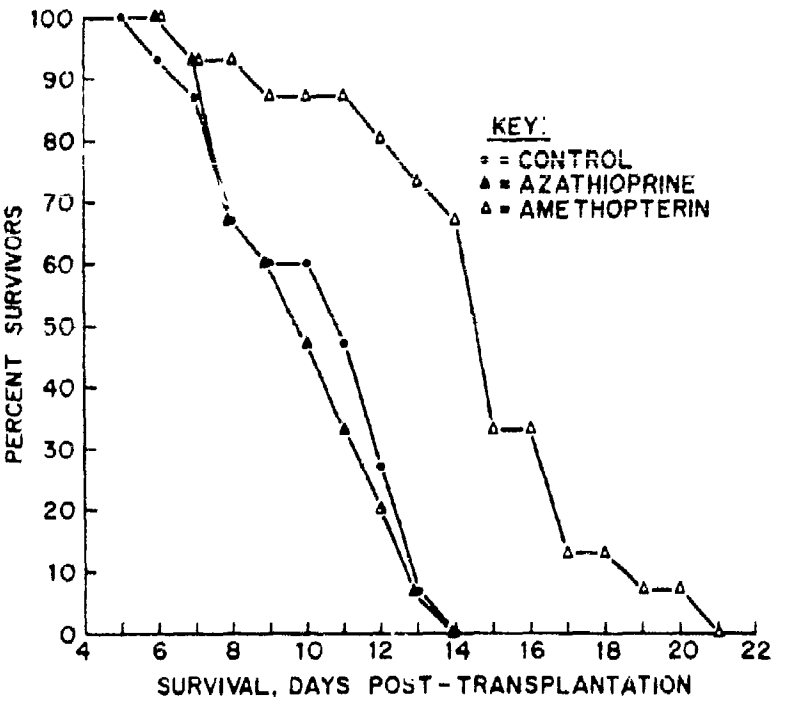

FIGURE 5

Survival after transplantation for the three experimental groups.

in the control group, 10.3 days in the azathioprine group, and 14.7 days in the amethopterin group. There is no significant difference between the average survival times of the control and azathioprine groups; however, the net effect of the amethopterin appeared to be simply one of forestalling the onset of secondary disease as the clinical course of this group paralleled the others after a brief delay. The survival times for the different blood types showed no statistically significant relationship with red cell types. In addition, an analysis of the number of cells received showed no significant difference among the treatment group means, indicating no bias in the survival times due to differences in the number of cells received. Comparison of the average survival times of the control and amethopterin groups yields $P < .001$.

Figure 6 shows the estimated degree of cellularity of vertebral bone marrow sections obtained at necropsy versus the day of death. Cellularity of vertebral marrow for normal monkeys in this age group in our colony is approximately 65% to 70%. Differing symbols have been used to indicate whether a take was obtained as defined by the peripheral blood findings. The control and amethopterin groups show similar pictures, with the latter shifted slightly in time corresponding to the increased survival times. The azathioprine group shows a marked reduction in cellularity as compared with the other groups. This finding was not unexpected since animals receiving autologous transplants with this azathioprine regimen showed evidence of marked bone marrow depression until administration of the drug was discontinued. Vertebral marrow sections of the 10 animals which did not receive marrow transplants appeared hemorrhagic or had less than 5% cellularity.

While the dose of radiation used in the present study is supralethal for animals in

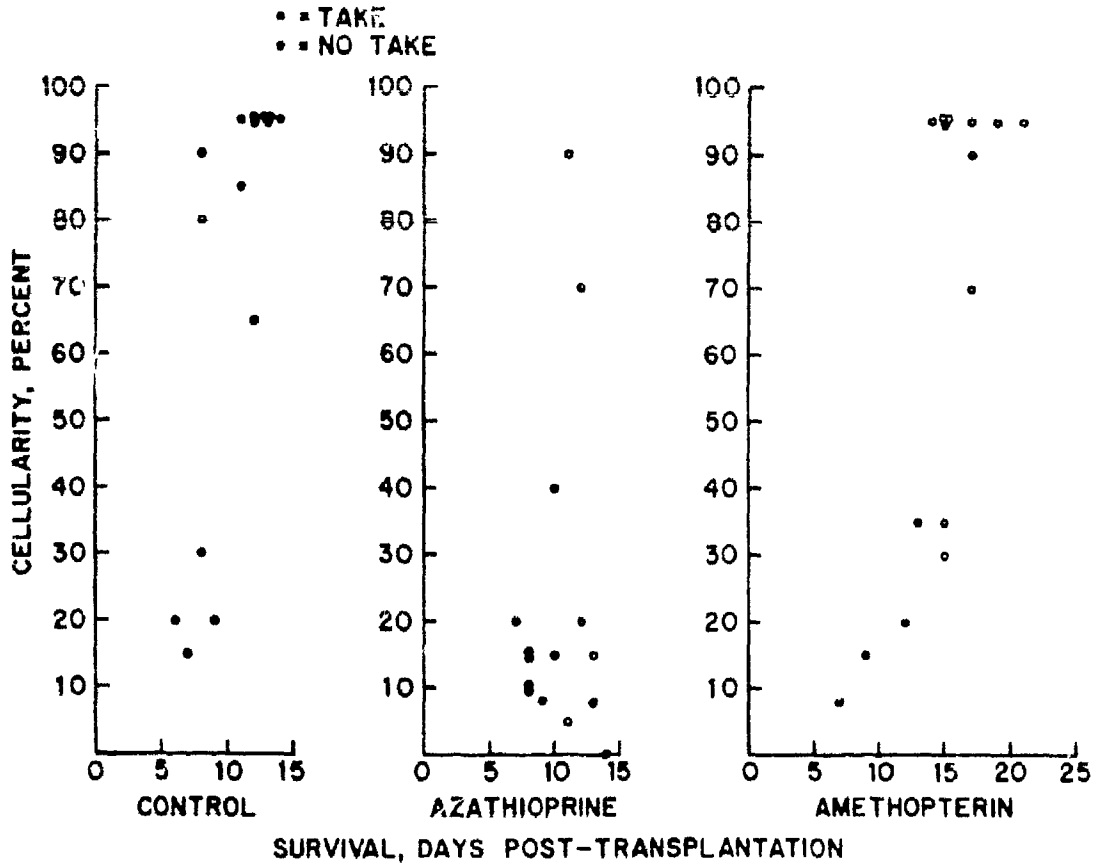

FIGURE 6

Relationship between vertebral marrow cellularity and day of death.

our colony, there were no early deaths in the nontransplanted group or in the animals treated with autologous bone marrow, suggesting that the dose used is below that which will cause a significant number of early deaths from gastrointestinal damage. An examination of the number of deaths occurring each day posttransplantation (fig. 7) shows that there were several early deaths in the control and azathioprine groups. Few deaths occurred during this period in the amethopterin group.

The number of animals is small, but the question may be raised as to whether the early deaths are the result of severe secondary disease or additional stress from rejection of the graft superimposed on the radiation damage. Crouch and Overman (9) have suggested that the decreased incidence of takes in their lethally irradiated, lower-dose groups may be due to rejection of the transplant by the host. In the present study this may be the mechanism for some of the early deaths in spite of a supralethal dose of radiation. If this were true, one might speculate that the potential action of an immunosuppressive agent is twofold: (1) suppression of the host's residual immune mechanisms with an overall increase in the number of takes; and (2) suppression of the graft-versus-host reaction. In the present study there was no evidence of an increased number of takes with the dose regimens utilized.

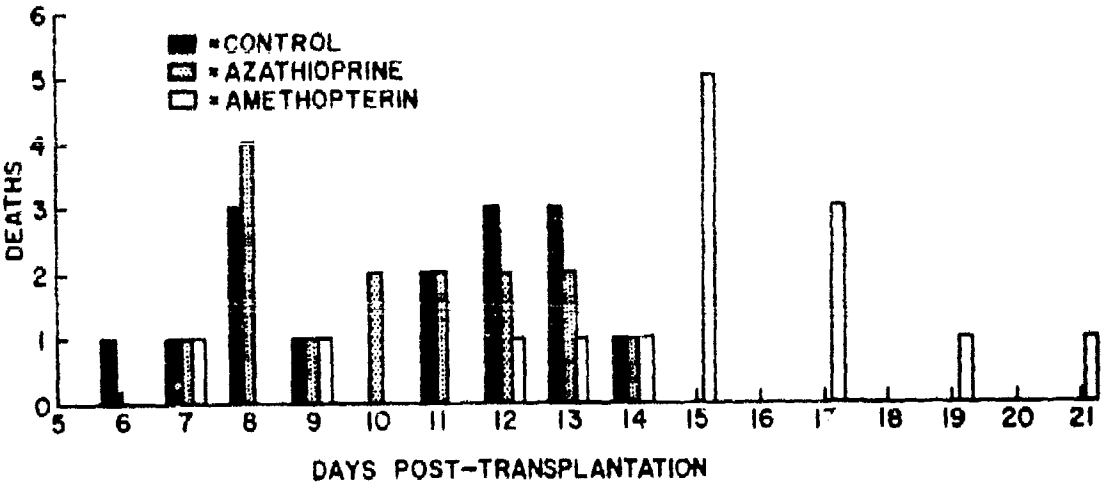

FIGURE 7

Number of deaths per day for the three experimental groups.

Although it is technically feasible to effect successful bone marrow transplants in man and other primates, the fatal secondary disease which intervenes remains an enormous obstacle to practical success. The potential usefulness of allogeneic bone marrow transplants in man—such as in leukemic patients, individuals with disseminated radiosensitive metastases, or victims of radiation accidents—remains a considerable stimulus to finding a successful method of overcoming secondary disease in man. Although Mathe and associates (16) and Beilby et al. (4) have had success in establishing chimerism in man, the overall results to date have been disappointing. Recently, evidence is accumulating that cyclophosphamide may be of some value in modifying secondary disease in primates (5, 11, 19), and it is possible that more effective immunosuppressive agents will appear in the future. Other possible approaches include antilymphocyte serums, better typing of histocompatability, the use of pooled marrow (16, 18), or modification of the immunologically competent cells by temporary storage of marrow under various conditions as suggested by Mathe et al. (17) and Van Bekkum (6). Combinations of these approaches may possibly prove effective.

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13. ABSTRACT

Forty-five rhesus monkeys received 980 R whole-body irradiation from a cobalt-60 source followed by allogenic bone marrow transplants. The animals were randomly divided into three groups: (1) bone marrow, no drug; (2) bone marrow, azathioprine; and (3) bone marrow, amethopterin. Supportive care was the same for all groups. Although there was a statistically significant prolongation of the average survival time in the group receiving amethopterin, the net effect was one of briefly delaying the onset of secondary disease as the clinical course of these animals ultimately paralleled that of the other groups. There was no significant difference in the average survival time of the azathioprine group as compared with the control group, and by the criteria cited there were fewer takes effected in the animals receiving azathioprine. The two drugs tested were essentially ineffective in the dose levels and regimens utilized.

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